Nick's Lab Notebook
iGEM 2015

6.22.15
To do
-Reconstitute primers
-PCR to create inserts with cutsites
Goals
Two PCR products using tdTomato as Template
1. LexA DBD + RFP-Transcription Factor
2. pFig2c + LexA DBD + RFP-Transcription Factor

> pFig2c is an alpha factor responsive promoter
>
> [LexA DBD + RFP-Transciption Factor] will be spliced into a plasmid backbone (pGEM22) which contains pAga1(alpha factor responsive promoter)

###[] Reconstituting primers
#126 >> LexA DBD FW (Xho1)
#127 >> LexA DBD RV (not1)
#128 >> pFig2c FW (Apa1)

###[] PCR of inserts "Fig" & "Lex"
Fig
0.5ul tdTomato (template)
2.5ul FW #128
2.5ul RV #127
1.5ul DMSO
18ul H2O
25ul phusion mm
Lex
0.5ul tdTomato (template)
2.5ul FW #126
2.5ul RV #127
1.5ul DMSO
18ul H2O
25ul Phusion mm

*Products ran on 1% agarose gel
> "fig" insert will contain pFig2c promoter ~ 2.7kb
> "lex" piece will have no promoter ~ 2kb

###[] Gel results
"fig" piece has correct band

"lex" piece not seen

6.23.15

###[] Digestion pFig2c part + plasmid backbone
pGEM22(plasmid)
10ul pGEM22
3ul cutsmart
16ul H2O
0.5ul Apa1
---------- RT for 1 hr
0.5ul Not1
---------- 37C for 1 hr
> pGEM22 contains pAga1

"Fig" (insert) >>> add to PCR product tube
5ul Cutsmart
0.5ul Apa1
---------- Rt for 1 hr
0.5ul Not1
---------- 37C for 1 hr

###[] New PCR "lex" piece from scratcg
"Lex"
0.5ul tdTomato (template)
2.5ul FW #126
2.5ul RV #127
1.5ul DMSO
18ul H2O
25ul Phusion mm

6.24.15
To do
-New PCR LexA DBD + RFP-TransCription Factor
-w/ & w/o DMSO

###[] PCR LexA DBD + tdTomato(RFP-TF)
w/ DMSO
0.5ul tdTomato (template)
2.5ul FW #126
2.5ul RV #127
1.5ul DMSO
18ul H2O
25ul Phusion mm

w/o DMSO
0.5ul tdTomato (template)
2.5ul FW #126
2.5ul RV #127
19.5ul H2O

```
                25ul Phusion mm

                        * Products ran on 1% agarose gel

###[] Gel results
        Visible bands in both PCR products at correct size
        (~2kb)

###[] Digest PCR products
        "Lex" and "Fig" parts gel extracted
        Plasmid Digest (pGEM22) was not cut

## 6.25.15 ##

> Hy130E used as a backup in case pGEM22 isn't cut
> both are pNH604 backbone, Hy130E is empty

###[] Plasmid Digests: Hy130E; pGEM22 ###
        Hy130E "A" ---> HyA
                10ul Plasmid
                3ul Cutsmart
                16ul H2O
                0.5ul Apa1
                ------------------ Rt for 1 hr
                0.5ul Not1
                ------------------ 37C for 1 hr

        Hy130E "X"---> HyX
                10ul Plasmid
                3ul Cutsmart
                16ul H2O
                0.5ul Xho1
                0.5ul Not1
                ------------------ 37C for 1 hr

        pGEM22 "A"
                10ul Plasmid
                3ul Cutsmart
                16ul H2O
                0.5ul Apa1
                ------------------ Rt for 1 hr
                0.5ul Not1
                ------------------ 37C for 1 hr

        pGEM22 "X"
                10ul Plasmid
                3ul Cutsmart
                16ul H2O
                0.5ul Xho1
                0.5ul Not1
```

------------------ 37C for 1 hr

###[] Gel Extraction Plasmid Digests ###
Both Hy130E strains cut correctly
pGEM22 not cut

###[] Ligation: [HyX + LexA DBD-RFP-TF] & [HyA + LexA DBD-RFP-TF] ###
HyA + Fig
2ul HyA
8ul Fig
1ul Ligase
2ul T4 Buffer
7ul H2O

HyA + Fig (negative control)
2ul HyA
1ul Ligase
2ul T4 Ligase
15ul H2O

HyX + Lex
1.5ul HyX
3ul Lex
1ul Ligase
2ul T4 Buffer
12.5ul

HyX + Lex (Negative Control)
1.5ul HyX
1ul Ligase
2ul T4 Buffer
15.5ul

*Samples kept at 16C overnight

6.26.15

[] Transformation: HyX & HyA Ligations
HyA ---> 1x, 8x
HyX ---> 1x, 8x

Plated on LB/CARB, left at RT over the weekend

6.29.15

###[] Colony PCR: HyX & HyA ###
HyX MM
70ul GoTaq
7ul FW #126
7ul RV #127

21ul H2O

HyA MM
70ul GoTaq
7ul FW #128
7ul RV #127
21ul H2O

6.30.15

###[] Colony PCR ran on 1% agarose gel ###
Faint bands from HyX colonies
No bands from HyA colonies

HyX colonies: 1,2,4,5,6
---> made into liquid cultures
> liquid cultures were taped to the side of the shaker for some odd reason..
> lost colonies 1,4 liquid cultures

###[] Back up Ligation Re-do ###

HyA
2ul T4 buffer
2ul HyA
8ul Fig
1ul ligase
7ul H2O

HyA (negative control)
2ul HyA
2ul T4Buffer
1ul Ligase
15ul H2O

###[] Colony PCR: 8 new colonies picked from HyA plate ###

9x mm
90ul GoTaq
9ul FW #126
9ul RV #128
27ul H2O
*looking for 3kb PCR product
> sample ran on 1% agarose gel

> colony 8 contains band

7.1.15

Miniprep liquid cultures

Nanodrop results

- HyA colony 8: 139.6 ng/ul
- HyX colony 5: 474 ng/ul
- HyX Colony 6: 699.6 ng/ul
- HyX colony 2: 549.9 ng/ul

7/2/15

Colony PCR HyX 8x plate
9x mm
90ul GoTaq
9ul FW #126
9ul Rv #127
27ul H2O
Ran on 1% agarose gel

7/8/15

Gel Extraction new HyA & HyX Digests
Gel fell apart-->faulty agarose gel stock?

New Digests

Hy130E "A" ---> HyA
10ul Plasmid
3ul Cutsmart
16ul H2O
0.5ul Apa1
---------- Rt for half day
0.5ul Not1
---------- 37C for overnight

Hy130E "X"---> HyX
10ul Plasmid
3ul Cutsmart
16ul H2O
0.5ul Xho1
0.5ul Not1
---------- 37C for overnight

7.9.15

Gel Extraction HyA & HyX Digestion

- HyX
  - cut correctly
- HyA
  - cut correctly

Ligation